J Lung Cancer 2012;11(2):71-76
http://dx.doi.org/10.6058/jlc.2012.11.2.71

MDA-7/IL-24 Expression and Its Relation with Clinicopathologic Factors in Lung Adenocarcinomas of 3 cm or Less in Diameter

Purpose: The melanoma differentiation-associated gene-7 (MDA-7) protein, also known as interleukin 24 (IL-24), is a novel candidate of tumor suppressor that has been found to experimentally induce apoptosis and growth inhibition in a variety of human malignant cells. However, there have been few studies about its role in lung adenocarcinoma. Even at the same stage and with similar pathologic characteristics, lung adenocarcinomas with a diameter of 3 cm or less can have a variable prognosis depending on their biologic characteristics. The purpose of this study is to define the relationship between MDA-7/IL-24 expression and the progression of small-sized lung adenocarcinomas. **Materials and Methods:** We performed immunohistochemical detection of MDA-7/IL-24 in forty-seven tissue samples from primary lung adenocarcinomas of ≤3 cm in diameter by using tissue microarray. **Results:** MDA-7/IL-24 immunoreactivity was observed in 20 (42.6%) of the 47 adenocarcinoma cases. MDA-7/IL-24 expression was positive in 66.7% of the adenocarcinomas ≤2 cm, and in 31.3% of the adenocarcinomas >2 cm or ≤3 cm in diameter. A statistically significant association was found between MDA-7/IL-24 expression and tumor size (p=0.03). Although this difference did not reach statistical significance, tumors with a negative MDA-7/IL-24 expression tended to more frequently show lymph node metastasis (p=0.07). There were no significant associations for other clinicopathologic characteristics. **Conclusion:** These results suggest the possible involvement of MDA-7/IL-24 in the growth and progression of small-sized lung adenocarcinoma. MDA-7/IL-24 immunoreactivity could be used to identify a subset of adenocarcinomas of the lung of 3 cm or less in diameter that have different biologic behavior. **(J Lung Cancer 2012;11(2): 71−76)**

Key Words: Interleukin-24, Adenocarcinoma, Lung, Immunohistochemistry

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Received: November 17, 2012
Revised: November 27, 2012
Accepted: November 27, 2012

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INTRODUCTION

Lung cancer is a major cause of cancer deaths. Among the histologic types of lung cancer, adenocarcinoma is becoming the most common in a majority of countries including Korea (1). Many types of small adenocarcinomas can now be detected as a result of advances in diagnostic methods. Despite their small size, however, some of these will already be advanced tumors at the time of diagnosis, or they may develop distant metastasis after complete surgical resection (2). Furthermore, lung adenocarcinomas are histologically heterogeneous with their characteristic patterns being lepidic, acinar, papillary, micropapillary, and solid (3). Given the histological and biological heterogeneity of adenocarcinomas, it is difficult to predict patient outcomes. Although several well-known biological markers have been reported, it is important to detect further biological markers of this tumor so as to develop appropriate treatment strategies and reliable estimates of the probability of patient survival.

Melanoma differentiation associated gene-7 (MDA-7), also known as interleukin 24 (IL-24), is a novel candidate of tumor

suppressor associated with differentiation, growth and apoptosis (4). MDA-7/IL-24 was discovered using a subtraction hybridization approach by exposing melanoma cells to terminal differentiation agents including interferon-ß and mezerein (4,5). Ectopic transfer of *MDA-7/IL-24* has been shown *in vitro* to suppress growth and induce apoptosis in a variety of human tumor cell lines, but similar effects have not been elicited in normal cells (6,7). MDA-7/IL-24 is believed to be associated with the induction of key molecules, thereby altering the balance between the pro- and anti-apoptotic proteins that mediate growth inhibition and apoptosis in several tumor types (8). As such, MDA-7/IL-24 seems to function as a novel tumor suppressor, and there is interest in the potential of *MDA-7/IL-24* gene transfer as cancer therapy. However, only one study has documented the clinical significance of MDA-7/IL-24 expression in non-small cell lung cancer (NSCLC), with positive MDA-7/IL-24 expression being found to be a significant factor for predicting favorable prognosis in adenocarcinoma (9).

Taking into account the importance of MDA-7/IL-24 in lung adenocarcinoma found in this earlier study, we performed immunohistochemical detection of MDA-7/IL-24 protein in lung adenocarcinoma tissue samples that had a maximal tumor diameter of 3 cm or less by using tissue microarray (TMA). The purpose of the current study is to define the relationship between MDA-7/IL-24 expression and the progression of small-sized lung adenocarcinoma. This is the first study to determine the clinicopathological role of MDA-7/IL-24 expression in Korean lung adenocarcinoma patients.

MATERIALS AND METHODS

1) Patients and tissue samples

A total of 47 formalin-fixed, paraffin-embedded tissue samples were obtained from Korean patients who underwent surgical resection for primary lung adenocarcinoma with a maximal tumor diameter of 3 cm or less at Dong-A University Medical Center from 2000 to 2005. No preoperative chemotherapy or radiotherapy had been performed in any of the subjects. Standard lobectomy and lymph node dissections were performed in every case. Cases in which any other malignancy had occurred before or after the primary lung cancer were excluded. Hematoxylin and eosin-stained slides of each case were reviewed to confirm the original diagnosis, based on the revised 2004 World Health Organization classification (10). The percentage of each histologic component (lepidic, acinar, papillary, micropapillary, and solid) was recorded. After estimating the amounts of each histologic subtype in increments of 5%, the tumors were classified based on their predominant growth pattern as proposed by the International Association for the Study of Lung Cancer, American Thoracic Society and the European Respiratory Society (3). The seventh edition of the American Joint Committee on Cancer tumor-node-metastasis (TNM) staging system was used for postoperative pathological staging (11). Clinical records, pathological reports and follow-up information were also obtained for every patient. The Institutional Review Board at Dong-A University Medical Center approved our study, and written informed consent was obtained from all patients for surgery and to use their resected samples for research.

2) Construction of the TMA

One millimeter cores were removed from the representative predominant histologic subtype of the adenocarcinomas, which had been previously formalin-fixed and paraffin-embedded. For all arrays, three cores of different areas of the representative tumor were removed from each sample and put in a new blank recipient paraffin block, and 4 μm-thick sections were taken for all the immunohistochemical staining. Full cross-sections from the paraffin blocks were used for five of the adenocarcinomas along with the adjacent normal lung tissue to confirm the staining patterns seen on the TMA.

3) Immunohistochemistry

Immunohistochemical staining for MDA-7/IL-24 was performed on the TMA slides by using the avidin-biotin-peroxidase complex method. Deparaffinization of all the sections was performed through a series of xylene baths, and rehydration was performed with a series of graded alcohol solutions. To enhance the immunoreactivity, microwave antigen retrieval was performed at 750 W for 30 minutes in Tris-ethylenediaminetetraacetic acid (pH 9.0). After blocking the endogenous peroxidase activity with 5% hydrogen peroxidase for 10 minutes, incubation with the primary antibody was performed for 1 hour at room temperature. The primary antibody used in immunostaining was rabbit polyclonal antibody directed against MDA-7/IL-24 (sc-12408; Santa Cruz Biotechnology, Santa Cruz, CA,

USA) used at a ratio of 1 : 200. An Envision Chem detection kit (DakoCytomation, Carpinteria, CA, USA) was used for the secondary antibody at room temperature for 30 minutes. After washing the tissue samples in Tris buffered saline for 10 minutes, 3,3'-diaminobenzidine was used as a chromogen, and Mayer's hematoxylin counterstain was then applied.

4) Immunohistochemical assessment

MDA-7/IL-24 immunoreactivity was defined as a sample showing a cytoplasmic staining pattern of the tumor tissue with minimal staining background. The percentage scoring of the immunoreactive tumor cells was as follows: 0 (0%), 1 (1～10%), 2 (11～50%), and 3 (＞50%). The staining intensity was visually scored and stratified as follows: 0 (negative), 1 (weak, blush/faint), and 2 (strong, obviously positive at ×20 magnification). A final score was obtained for each case by multiplying the percentage and the intensity score. Therefore, tumors with a multiplied score exceeding 4 (i.e., the tumors with a strong intensity in ＞10% of tumor cells) were recorded as having positive immunoreactivity for MDA-7/IL-24; all other scores were considered to be negative.

5) Statistical analysis

Associations between MDA-7/IL-24 expression and the clinicopathologic characteristics of the tumors were analyzed using the χ^2 test or Fisher's exact test. A p-value of less than 0.05 was considered statistically significant. All statistical tests were performed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

1) Clinicopathologic characteristics

Patients consisted of 25 men and 22 women, aged from 40 to 74 years (median, 58 years). The tumor size ranged from 1 to 3 cm, with fifteen cases involving tumors ≤2 cm and 32 cases involving tumors ＞2 cm. When classifying the tumors according to their major histologic components, the most common subtype was acinar-predominant (31.9%), followed by solid-predominant (25.6%), lepidic-predominant (21.3%), papillary-predominant (19.1%), and micropapillary-predominant (2.1%). There were 26 negative cases and 21 positive cases for lymphovascular invasion. There were 28 negative cases and 19 positive cases for lymph node metastases. According to the TNM staging system, 28 patients were stage IA, 18 were stage IIA, and one was stage IIIA at the time of surgery.

2) Immunohistochemical findings

All the tumor cores demonstrated similar staining characteristics. Six cases had only two cores with adequate tissue to carry out evaluation. The staining patterns of the TMA cores showed results that were concordant with those for the five full

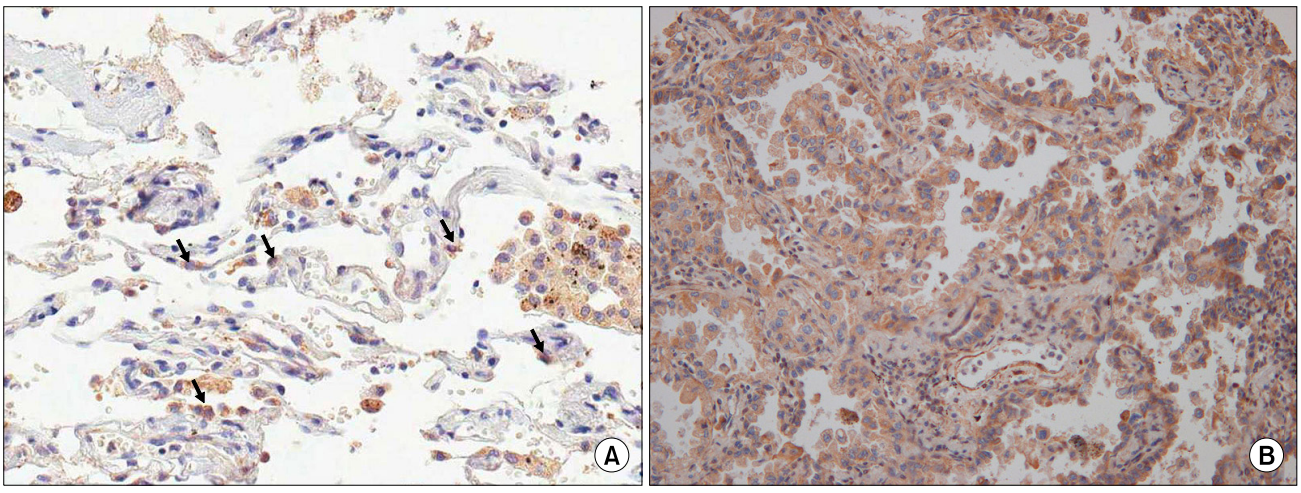

Fig. 1. Immunohistochemical staining of melanoma differentiation-associated gene-7 (MDA-7)/interleukin 24 (IL-24). The expression of MDA-7/IL-24 protein was detected in the cytoplasm of both normal and tumor cells. (A) In the normal lung, the nonneoplastic alveolar epithelial cells (arrows) and macrophages were occasionally reactive for MDA-7/IL-24 (×100). (B) In the tumor tissue, MDA-7/IL-24 immunoreactivity was observed in 20 (42.6%) of the 47 adenocarcinoma cases (×200).

cross-sections. The expression of MDA-7/IL-24 protein was detected in the cytoplasms of both the normal cells and the tumor cells. In the adjacent normal lung from the five full cross-sections, the nonneoplastic alveolar epithelial cells and macrophages were occasionally reactive for MDA-7/IL-24 (Fig. 1A). In the tumor tissue, MDA-7/IL-24 immunoreactivity was observed in 20 (42.6%) of the 47 adenocarcinoma cases (Fig. 1B). Expression was limited to those tumor cells without any background labeling. The staining intensity and percentage were relatively well-concordant.

3) Correlation between MDA-7/IL-24 immunoreactivity and the clinicopathologic characteristics

The various clinicopathologic characteristics of the patients and their tumors were compared according to MDA-7/IL-24 immunoreactivity (Table 1). MDA-7/IL-24 expression was positive in 66.7% of the adenocarcinomas ≤2 cm, but in only 31.3% of the adenocarcinomas >2 cm or ≤3 cm in diameter. A statistically significant association was found between MDA-7/IL-24 expression and tumor size (p=0.03). Although this difference did not reach statistical significance, tumors with a negative MDA-7/IL-24 expression tended to more frequently show lymph node metastasis (p=0.07). There were no significant associations with age, gender, predominant histologic subtype, lymphovascular invasion, or stage.

Table 1. Correlations of the MDA-7/IL-24 Expression with the Conventional Clinicopathologic Characteristics of 47 Patients with Lung Adenocarcinoma of 3 cm or Less in Diameter

Clinicopathologic characteristics	MDA-7/IL-24 expression		p-value
	Negative (n=27)	Positive (n=20)	
Age, yr	58.8±7.1	58.5±9.0	0.61
Gender			0.13
Male (n=25)	17	8	
Female (n=22)	10	12	
Tumor size, cm			0.03
≤2 (n=15)	5	10	
>2 or ≤3 (n=32)	22	10	
Predominant histologic subtype			0.44
Lepidic (n=10)	4	6	
Acinar (n=15)	9	6	
Papillary (n=9)	7	2	
Micropapillary (n=1)	1	0	
Solid (n=12)	6	6	
Lymphovascular invasion			0.59
Negative (n=26)	14	12	
Positive (n=21)	13	8	
Lymph node metastasis			0.07
Negative (n=28)	13	15	
Positive (n=19)	14	5	
Stage			0.15
I (n=28)	13	15	
II (n=18)	13	5	
III (n=1)	1	0	

MDA-7: melanoma differentiation-associated gene-7, IL-24: interleukin 24.

DISCUSSION AND CONCLUSION

In the present study, we have shown the prevalence of MDA-7/IL-24 immunoreactivity in the adenocarcinomas of the lung that were 3 cm or less in diameter, and we found a significant correlation between MDA-7/IL-24 immunoreactivity and the tumor size. These results suggest the possible involvement of this protein in the growth and progression of small-sized lung adenocarcinoma.

MDA-7/IL-24, a novel tumor suppressor gene, is mapped within the IL-10 family cytokine cluster to 1q32.2-q41, and encodes a protein consisting of 206 amino acids, secreted in mature form as a 35～40 kDa phosphorylated glycoprotein (12). MDA-7/IL-24 is expressed by diverse cell types, including B cells, natural killer cells, dendritic cells, monocytes and melanocytes (6). MDA-7/IL-24 was first identified by subtraction hybridization from human melanoma cells and the expression of MDA-7/IL-24 was inversely related to human melanoma progression, being highest in melanocytes and lowest in metastatic melanomas (5-7).

Although its physiological role is poorly understood, multiple anticancer mechanisms of MDA-7/IL-24 have been reported, including cancer-specific apoptosis induction, cell cycle regulation, an ability to inhibit angiogenesis, potent bystander antitumor activity, and a capacity to enhance the sensitivity of tumor cells to radiation and chemotherapy (13,14). Forced expression of MDA-7/IL-24 in cancer cells results in irreversible growth inhibition, reversal of the malignant phenotype and terminal differentiation. Transfection of *MDA-7/IL-24* into melanoma cells reduces growth without a similar effect on normal cells and this antiproliferative activity of MDA-7/IL-24 has also been detected in a variety of cancer cells including those of the lung (15). In this study, we found a statistically significant inverse correlation between MDA-7/IL-24 expression and

tumor size, which is consistent with the antiproliferative activity reported by others (13-15). In addition to this antiproliferative effect in cancer, Ishikawa et al. (9) reported that positive MDA-7/IL-24 expression was a significant factor for predicting favorable prognosis in lung adenocarcinoma, which was confirmed by a multivariate analysis. However, our study also revealed that tumors with a negative MDA-7/IL-24 expression tended to more frequently show lymph node metastasis, but this difference did not reach statistical significance (p=0.07). Moreover, survival analysis was not performed in our study. Therefore, it is hard to conclude the prognostic significance of MDA-7/IL-24 expression in lung adenocarcinoma. Further work with a larger numbers of tumor samples is needed to test the possibility of using MDA-7/IL-24 as a suitable marker for metastatic potential or favorable prognosis in small-sized lung adenocarcinomas.

In addition to its prognostic utility, further mechanistic studies are warranted to explore the potential for the therapeutic manipulation of MDA-7/IL-24 in lung cancer. Enforced expression of *MDA-7/IL24*, by use of a recombinant adenoviral vector-mediated *MDA-7/IL-24* gene (Ad.mda-7), has been shown to inhibit the growth of a broad spectrum of cancer cells in vitro, without exerting deleterious effects in normal human epithelial or fibroblast cells (16,17). Ad.mda-7 mediates induction of the growth arrest and NDA damage (*GADD*) genes by means of the p38 mitogen-activated protein kinase pathway, thereby resulting in the selective induction of apoptosis in human cancer cells (18). Recent studies in lung carcinoma cells also have noted that Ad.mda-7 can act as a radiosensitizer (15). Furthermore, it has been reported that the treatment of erlotinib (a small-molecule epidermal growth factor receptor [EGFR] tyrosine kinase inhibitor) combined with Ad.mda-7 therapy revealed a significant increase in cell growth inhibition and apoptosis induction in human melanoma cells (19). In addition, combined treatment of NSCLC cells with an EGFR inhibitor, tarceva, and a Ad.mda-7 or a GST-tagged recombinant protein (GST-MDA-7) synergistically enhanced growth inhibition and apoptosis (20). These results suggest that a combination of MDA-7/IL-24-mediated molecular therapy and EGFR inhibitors may be a potential treatment strategy for lung adenocarcinoma patients. In this context, our study might serve as a basis for defining the role of MDA-7/IL-24 expression in lung adenocarcinoma so as to guide the development of combination treatment designs in future preclinical and clinical trials.

In conclusion, this study indicates that MDA-7/IL-24 immunoreactivity may identify the subset of adenocarcinomas of the lung that are 3 cm or less in diameter that have different potential for growth and progression. We hypothesize that MDA-7/IL-24 is lost during the dedifferentiation of tumor cells, suggesting that the lack of MDA-7/IL-24 could be associated with aggressive biologic behavior. Therefore, our results highlight the fact that among the various molecular events associated with a tumorigenic phenotype of lung adenocarcinomas, the level of the MDA-7/IL-24 expression should be also taken into account. Taken together, MDA-7/IL-24 may represent a new target for therapeutic intervention in a subset of lung adenocarcinoma patients.

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